Fisheries and Oceans Canada | Pêches et Océans Canada
Science | Sciences
National Capital Region
Canadian Science Advisory Secretariat
Science Advisory Report 2013/049

POTENTIAL EXPOSURE AND ASSOCIATED BIOLOGICAL EFFECTS FROM AQUACULTURE PEST AND PATHOGEN TREATMENTS: ANTI-SEA LICE PESTICIDES (PART II)

Green fluorescein dye, used to track pesticide transport and dispersal, being released from an Atlantic salmon net-pen following an anti-sea lice tarp bath treatment. (courtesy of Fred Page, DFO, St. Andrews Biological Station)

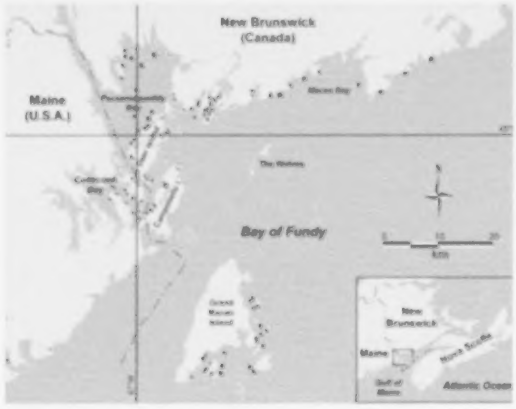

Figure 1. Map of southwestern New Brunswick, showing approved finfish farms in 2010.

Context:

Sea lice are naturally occurring marine ectoparasites that attach to the skin and feed on the mucus, blood and surface tissues of salmon and other species of fish. Sea lice species occur on both the east and west coasts of Canada; however, there are differences in sea lice behaviour and parasite-host population dynamics observed between these two environments. Minor sea lice infestations are generally not harmful, but damage to surface tissues can occur as sea lice abundance on the fish increases. Heavy sea lice loads have the potential to affect the fish's physiology, behaviour and increase the risk of death due to osmotic shock, blood loss or secondary infections. Controlling sea lice abundance on farmed salmon reduces production losses, maintains fish welfare and reduces the risk of increasing infestation levels on surrounding wild salmon and other aquatic resources. Generally, integrated fish health plans include measures to control sea lice abundance through husbandry practices (e.g., single year class stocking, and fallowing); the development and use of non-chemical control approaches (e.g., light traps, cleaner fish, etc.); and the use of chemotherapeutants (pesticides and drugs).

Peer-reviewed science advice on the potential exposure and biological effects of sea lice bath treatments on non-target organisms, including commercial fisheries resources (e.g., lobsters), has been requested to support the development and subsequent implementation of regulations under s 36(3) of the Fisheries Act to manage the use of chemotherapeutants to control fish pathogens and pests affecting farmed finfish and their release into the environment, and other management activities.

This Science Advisory Report is from the March 13-15, 2013 CSAS meeting on Guidelines on Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments: Anti-Sea Lice Bath Treatments (Part II). Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

Canada

SUMMARY

- During anti-sea lice pesticide treatments in southwest New Brunswick, fluorescein dye was mixed with the pesticide in order to trace the transport and dispersal of the pesticide. *In situ* water samples were analyzed for both the pesticide and fluorescein dye concentrations. This data shows a good relationship between *in situ* fluorescein dye concentration and *in situ* pesticide concentration indicating that the concentration of fluorescein dye is a good surrogate for field pesticide concentrations, over time periods of a few hours.

- Comparison of the horizontal spread of dye released from tarp pesticide applications to the Okubo model (1971, 1974) indicates that the Okubo model underestimates pesticide dilution from net-pens over the first few hours after release. However, a modified Okubo model based-approach that takes into consideration the initial enhancement of the horizontal spread (assumed to be due to cage infrastructure) gives a more accurate estimation of dye plume size and dilution (concentration).

- The characteristics of the immediate discharge from well-boats seem to be reasonably well represented by jet dynamic steady state theory. Based on this theory, entrainment of water into the effluent should result in pesticide dilution up to a factor of 40 at a distance of 100 m from the discharge point, based on a 0.5 m discharge pipe diameter.

- Estimates of effects thresholds for American lobsters and other indigenous invertebrates, based upon the predicted exposure profile, the prescribed treatment concentrations and the concentration that is lethal to 50% of the test organisms (LC_{50}) after 1 hour pesticide exposure, indicate that the potential magnitude of effects increases from Interox-Paramove® 50 (active ingredient: hydrogen peroxide) to Salmosan® 50WP (active ingredient: azamethiphos) to Excis® (active ingredient: cypermethrin) to AlphaMax® (active ingredient: deltamethrin). Sensitivity is species and life stage dependent for all four pesticides tested.

- Of the four pesticides studied, there is considerably more toxicology data available for Salmosan® 50WP.

- Sublethal and delayed effects were observed in adult American lobsters after repeated pulse exposures to Salmosan® 50WP at 10% and 5% of prescribed treatment concentrations, but no sublethal or delayed effects were observed at 1% or 0.1% of prescribed treatment concentration.

- In order to give an indication of the potential risks associated with exposure to each of the four anti-sea lice pesticides at *in situ* concentration and exposure durations, risk quotients (RQ) were calculated using the ratio of the exposure concentration to the 1-ho· · LC_{50} concentration. Based on the most sensitive non-target species tested, the pesticide that has the lowest risk quotient at the tarped net-pen treatment concentration (i.e., the concentration initially released into the environment) is: Paramove® 50 (RQ = 1.2), followed by Salmosan® 50WP (3.1), Excis® (151) and AlphaMax® (588).

- Risk quotients calculated for the concentration at discharge after well-boat treatments, for the most sensitive species tested, based on 1-hour LC_{50}s, ranged from: 0.46 for Paramove® 50, to 1.16 for Salmosan® 50WP, and 221 for AlphaMax®.

- Risk quotients calculated at 10 m from the discharge pipe (following well-boat bath treatments), based on 1-hour LC_{50}s, ranged from: 0.1 for Paramove® 50, 0.3 for Salmosan® 50WP, and 55 for AlphaMax®. Similarly, the estimated risk quotient at 100 m from the point of well-boat discharge ranged from 0.01 for Paramove® 50, 0.03 for Salmosan® 50WP and 6 for AlphaMax®.

- Based on the relative calculated risk quotients, the predicted pelagic area of influence (i.e., the predicted plume area at or above LC_{50} following release of the pesticide) following well-boat treatment is estimated to be smaller than the area of influence following tarp treatment (i.e., 1100 m^2 for Salmosan® 50WP to be diluted to LC_{50}, following tarp treatment, and 50,000 m^2 for AlphaMax®).

- Further validation and refinement to enhance the models would be beneficial, particularly with respect to vertical mixing and exposure of benthic environments, sub-lethal or no effect level (NOEC) interactions, and population and lifestage considerations for other non-target species. This is particularly important for application of the approach to other salmon producing areas in Canada.

- Although the field work was conducted in southwest New Brunswick, the general principles regarding transport and dispersal and the orders of magnitude of dilution are expected to apply elsewhere since the Okubo relationship is based on data collected from many places around the world. The modified Okubo-model should be tested to validate the effect of farm infrastructure on horizontal spread and dilution rates. To apply the Okubo models elsewhere and to improve their predictability, local oceanographic and environmental data are required for model parameterization.

- Site-specific differences including local hydrography, bathymetry, treatment procedures, local stratification and current regime will influence the depth to which pesticides mix and the direction and magnitude of the pesticide transport. The bathymetry within the zones of influence will also influence whether the benthic habitat will be exposed.

- While the risk quotient approach to estimating the potential exposure concentrations of pesticides to non-target organisms can be applied across all salmon farming regions in Canada, characterization of the effects requires local knowledge of the biology, ecology, and population dynamics of non-target species.

- The approach developed predicts the potential for effects to individuals but does not address population scale effects.

INTRODUCTION

Salmon aquaculture in Canada is one of several maritime activities, and while the regulation of marine aquaculture varies across Canada, best practices from each region can help to inform management considerations in other regions.

Farmed salmon are introduced into net-pens disease and parasite-free. However, as in other animal food production systems, it may become necessary to treat the aquaculture species for diseases, parasites and fouling organisms that are endemic to the culture area. Generally, integrated fish health plans include measures to control sea lice abundance through husbandry

practices (e.g., single year class stocking, and fallowing); management practices; and the use of chemotherapeutants (pesticides and drugs). The use of non-chemical control approaches is in development (e.g., light traps, cleaner fish, etc.). Although management and husbandry practices have evolved over the past 20 years, aquaculture operators still rely on the use of pesticides and drugs (chemotherapeutants) to combat infestations of ecto-parasites, such as sea lice. In the southwest New Brunswick salmon aquaculture industry and elsewhere in Canada and the world, it is sometimes necessary to control sea lice abundance on cultured Atlantic salmon using chemotherapeutants. Controlling sea lice abundance on farmed salmon reduces production losses, maintains fish welfare and reduces the risk of increasing infestation levels on surrounding wild salmon and other aquatic resources.

Chemotherapeutant use is regulated in Canada, and can only be used under prescription from a licensed veterinarian. The Food and Drugs Act, the Pest Control Products Act, the Canadian Environmental Protection Act, 1999, and the Fisheries Act (s. 36) are the four key federal legislative tools for regulating fish pathogen and pest treatments.

Within the regulatory framework, chemotherapeutants used in the aquaculture industry are classified as either a drug or a pesticide based upon their application method. Generally, products applied topically, or directly into water, are considered pesticides, while products delivered through medicated feed or by injection are considered drugs.

Since 2009, three different anti-sea lice pesticides have been temporarily registered for use in aquaculture in Atlantic Canada for various periods of time through the Pest Control Products Act Emergency Registration provision. These were: Interox-Paramove® 50 (active ingredient (a.i.): hydrogen peroxide); Salmosan® 50WP (a.i.: azamethiphos); and AlphaMax® (a.i.: deltamethrin). Whereas in 2011 and 2012, only the pesticides Interox-Paramove® 50 and Salmosan® 50WP were available for use (through Emergency Registrations).

Pesticide bath treatments can be conducted in one of three ways: tarping or skirting of the salmon net-pens, or using well-boats. Tarp and skirt bath treatments involve decreasing the volume of water in a salmon net-pen by either completely enclosing the net-pen in an impervious tarp (tarping), or by surrounding the net-pen with a series of impervious tarps to a depth below that of the bottom of the pursed net without enclosing the bottom of the net-pen (skirting). The prescribed amount of pesticide to achieve treatment concentration is then added to the net-pen for the recommended time period, at which point the skirt or tarp is removed and the treatment water is released. Well-boat treatments are conducted by pumping cultured salmon into treatment chambers, or wells, in specially designed boats. The pesticide is then added to the well for the prescribed time. Following treatment the treatment water is discharged from the well into the surrounding water, while the well is simultaneously flushed with fresh seawater. Following flushing, the fish are then pumped back into the net-pen.

The total amount of pesticide required to treat a single net-pen of fish is dependent on the application method (i.e., tarp/skirt/well-boat), volume (i.e., net-pen or well size), and for well-boat treatments, the biomass of the fish to be treated (i.e., the number of well-boat applications required to treat a single net-pen is dependent on the size of the treatment well, and the size and number of fish in the net-pen). As an example, a 70-m circular tarped net-pen is estimated to have a treatment volume ranging from 1500 m^3 - 3000 m^3. In southwest New Brunswick, one of the well-boat is configured with two 330 m^3 wells. To achieve the same treatment concentration, the quantity of pesticide required for a tarped net-pen is 2-4.5-times greater than the quantity required to achieve the same treatment concentration within two 330 m^3 wells.

However, treating all fish in a single net-pen may require multiple well-boat treatments and additional pesticide, resulting in multiple pesticide discharges and exposure profiles. The exact number of well-boat treatments per net-pen will depend on the well volume in the well-boat and the biomass of fish to be treated.

In order to estimate the potential for exposure and biological effects on non-target organisms associated with the use of anti-sea lice pesticides, research and analysis has been undertaken. The purpose of this science advisory process was to examine the knowledge base regarding: (1) the fate and transport dynamics, dispersion and dilution following pesticide treatments, and dispersion models for estimating potential exposure concentration and zone, post-treatment; (2) the characterization of the lethal and sub-lethal toxic effects of four anti-sea lice pesticides on key indigenous non-target organisms using laboratory toxicity testing; (3) the potential for indigenous non-target organisms in the environment to be exposed to biologically relevant concentrations of anti-sea lice pesticides post-treatment; and (4) an analysis of the applicability of the dispersion models and toxicity studies to other salmon farming areas in Canada and to other non-target species.

ANALYSIS

1) Transport and dispersal of anti-sea lice pesticides post-treatment

To assess the factors affecting the location and magnitude of exposure of non-target organisms to anti-sea lice pesticides following bath treatment, the transport and dispersal of pesticides post-treatment was studied by adding fluorescein dye to commercially applied pesticide treatments.

There is a relationship between the concentration of azamethiphos (active ingredient in Salmosan® 50WP) or deltamethrin (active ingredient in AlphaMax®) and the fluorescent dye in water samples from within the released treatment (discharge) water (Figure 2). There is only limited data for establishing the relationship between dye and deltamethrin due to the limited number of commercial AlphaMax® treatments conducted. A similar relationship between hydrogen peroxide and dye could not be established due to interactions between hydrogen peroxide and the florescent dye that interfere with the ability to measure hydrogen peroxide concentration. However, it is considered reasonable to assume that the dye can be used as a surrogate for hydrogen peroxide, at least over the short time scales considered (a few hours).

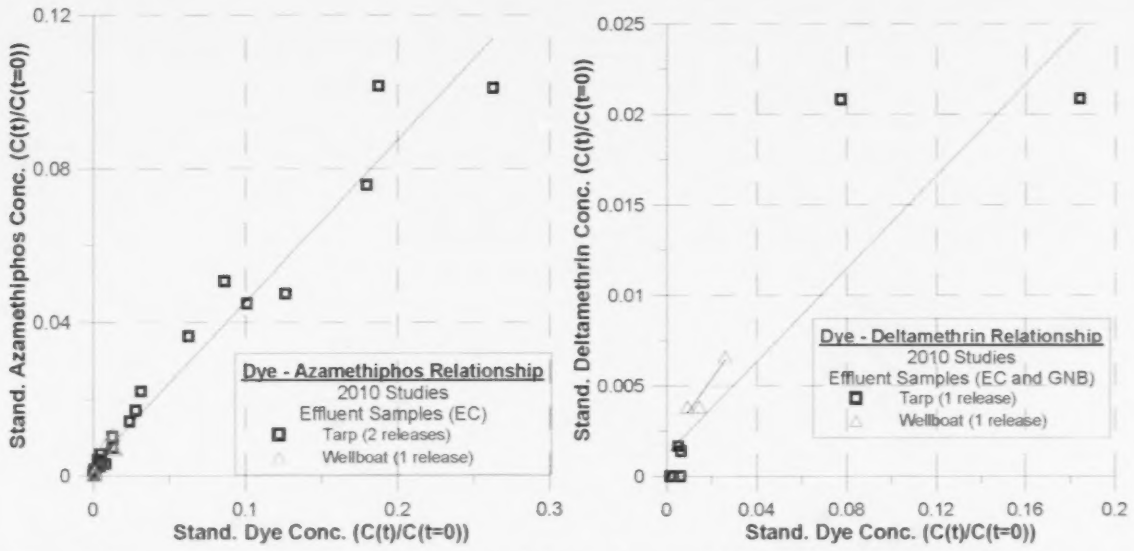

Figure 2: Linear relationship between fluorescent dye and azamethiphos (left graph) and deltamethrin (right graph) concentrations in effluents following salmon aquaculture treatments. Dye concentrations were standardized to the initial concentration. Black open squares are effluent samples from tarp releases (two releases for the dye-azamethiphos relationship, and one release from the dye-deltamethrin relationship). Red open triangles are from well boat releases (one release each for the two dye-pesticide relationships). Effluent samples from azamethophos treatments were obtained from Environment Canada. Effluent samples from deltamethrin treatments were obtained from Environment Canada and the Government of New Brunswick.

Release from net-pen after application by skirt or tarp

Mixing of the dye/pesticide with the water in the net-pen takes time and the dye/pesticide may not be equally dispersed within the net-pen by the end of the treatment time. The extent of mixing can be influenced by the application method. Upon removal of the tarp or skirt, the flushing time of the dye/pesticide from the net-pen can range from about 7 minutes to about 2.5 hours (Figure 3). Flushing time is influenced by environmental, husbandry, biophysical and operational factors. The released dye/pesticide effluent forms an elongated plume. Regardless of initial mixing, the concentration of dye/pesticide is not uniform within the plume.

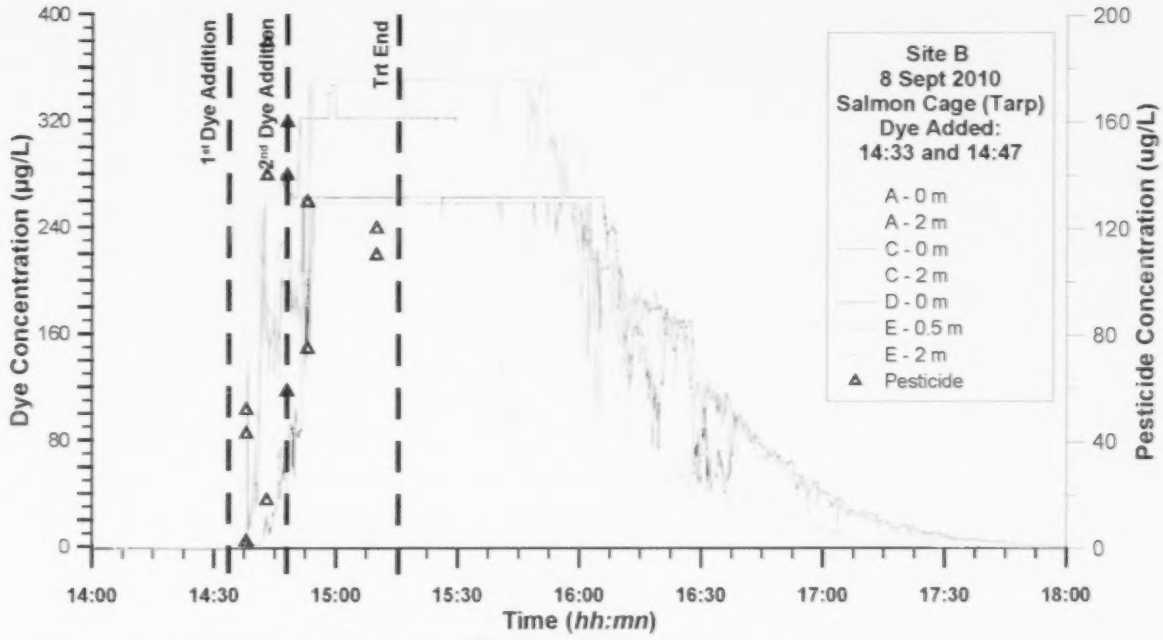

Figure 3: An example of dye and pesticide concentration inside a treated (tarped) net-pen. Dye concentrations (solid lines) plateau during treatment because dye concentrations exceed the detection limit of the fluorometers. Pesticide concentration (open triangles) varies during the treatment time, indicating that the pesticide is not equally mixed within the tarped net-pen. The first two dashed vertical lines represents the time when dye was added to the tarped net-pen, the third vertical dashed line indicates when the tarps were removed from the net-pen, ending the treatment. The dye concentration exceeds the detection limit of the fluorometers for the first 30-45 minutes following the removal of the tarp, and then decreases until it is no longer detectable inside the net-pen 2-2.5 hours following the end of the treatment. Fluorometers were placed both at the surface and 2 metres below the surface.

Upon release of dye/pesticide from a net-pen, the movement of the effluent is driven by two separate processes: advection and dispersion. Advection is the process of movement of the centre of mass (horizontal and vertical) due to currents, while dispersion is how the pesticide becomes mixed and diluted around that centre of mass, through turbulence, and velocity shears.

Effluent plumes tend to be elongated in the direction of transport. The observed horizontal diffusion rates are quite variable, but consistent with literature values. Using an elliptical approximation, the major axis (Kx) values ranged from 0.65 - 7.6 m^2s^{-1} and the minor axis (Ky) values ranged from 0.04 - 0.48 m^2s^{-1}.

Effluent plumes can travel up to several hundred metres within an hour, up to 1.5 kilometres within two hours, and up to 2 kilometres within three hours post-release. The distance effluent plumes travel is dependent on currents, tides and local vertical and horizontal boundaries. Tarp-applications tend to be undertaken in lower current conditions for operational reasons, although changes in currents can occur once application has started.

Vertical mixing varies between sites and treatments, although the maximum depth of dye and pesticides measured post-release was less than 25 metres for skirt and tarp treatments.

Net-Pen Treatment: Dispersal Models

The use of dispersal models to predict the transport and dispersal of pesticides from net-pens after application was evaluated. It is important to note that models are representations of reality using approximations and simplifications, and as such contain uncertainties and are not an exact recreation of reality. Therefore, although model outputs need to be considered together with empirical data they are useful tools to study the effects of different conditions and hence provide the ability to study various scenarios.

There is good agreement between the observed dispersal of dye/pesticide and the dispersal predicted using the Okubo (1971, 1974) model when no nets or fish in cages are present. With the presence of aquaculture infrastructure (cages, nets, fish, etc.) the initial dispersion of the effluent occurs more quickly than predicted by the Okubo model; however, once the dispersal has spread beyond the farm infrastructure, the plume disperses in a manner consistent with the Okubo model. A modification to the Okubo model that takes into account the farm infrastructure improves agreement between the model and observed dispersal at aquaculture sites. The Okubo model only predicts the area of the plume, not the shape.

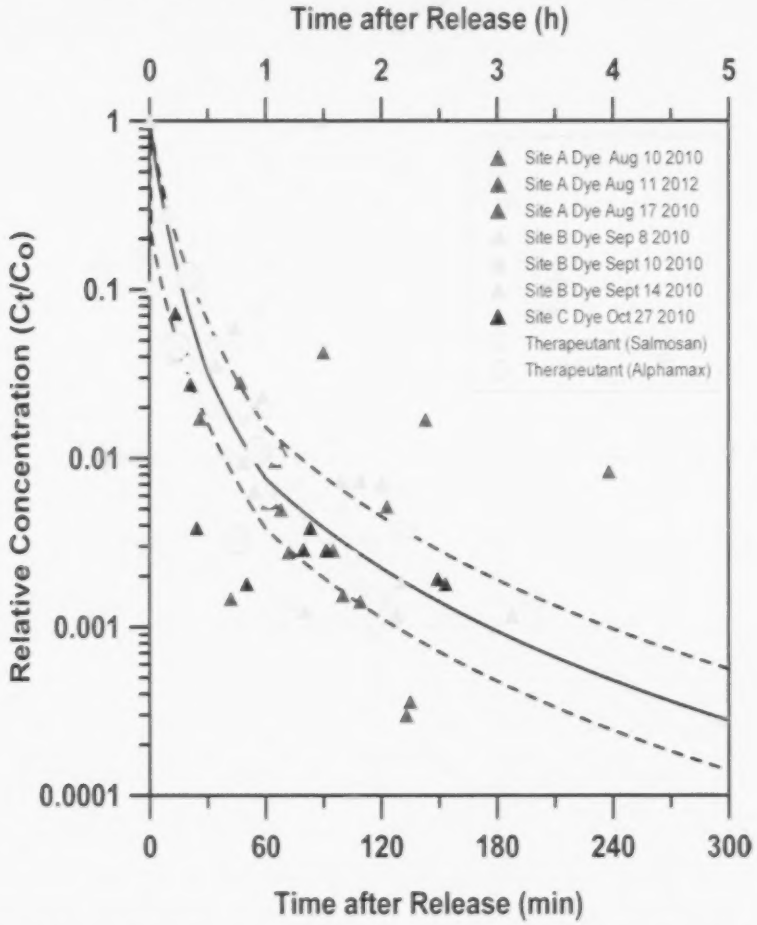

Figure 4: Modified Okubo model estimates of the temporal decrease in the standardized dye (triangles) or pesticide (circles) concentration after release from a tarped net-pen.

An implementation of the Finite Volume Coastal Ocean Model (FVCOM) for southwest New Brunswick predicts the general magnitude and rate of dilution of the effluent plume reasonably well, although it may underestimate the dilution over longer time scales. The FVCOM model predicts the direction and magnitude of the effluent plume transport as well as its shape and dilution.

While the dispersal of pesticide can be modeled using Okubo and FVCOM models, there are differences between the model predictions and the observed dispersal of dye upon release from net-pen sites following tarp or skirt applications. The Okubo model only predicts the area of the plume but not shape. For FVCOM, the differences range from the shape of the plumes, direction of drift, and the magnitude of plume displacement. The direction of the differences between FVCOM model predictions and observed values were not consistent. However, the overall exposure envelopes generated by the model, over all tidal phases, encompasses the majority of the observed areas exposed during dye/pesticide release studies.

Net-Pen Treatment: Factors Influencing Exposure

The factors influencing the concentration of pesticide released post-tarp treatments include the:

- size of the tarped net-pen (e.g., diameter, volume);
- amount of the pesticide used;
- mixing within the bath containment volume;
- proximity and nature of nearby net-pens and other farm infrastructure;
- net mesh size and degree of bio-fouling (porosity); and
- treatment procedures (e.g., how tarps are removed, pursing and dropping of nets).

Environmental factors also influence the dilution of pesticide following tarp or skirt bath treatments, such as the:

- rates of mixing in the horizontal and vertical dimensions;
- rates of horizontal advection;
- spatial variation in the flow field;
- vertical stratification;
- local bathymetry that determines whether the sea bottom and inter-tidal areas are near enough to be at risk of exposure;
- weather, wind and waves; and
- water quality characteristics such as temperature, salinity, pH, dissolved oxygen content, suspended organic and sediment loads that may influence the chemical behaviour of the pesticide in the ambient water.

Release from Well-boats

For pesticide treatments using well-boats, the pesticide (and when used, dye) is injected into the treatment well, and becomes mixed within the well during the treatment duration. Post-treatment, the effluent is pumped out, while fresh seawater is pumped into the well, resulting in a decrease in the concentration of dye/pesticide in the discharge plume over time. The pumping of the effluent creates a jet stream that mixes with the surrounding water, resulting in further dilution of pesticide through entrainment of water into the plume (Figure 5). Once the effluent has been fully discharged from the boat, the transport and dispersal of the resulting

effluent plume can be estimated using the same techniques as used for tarp or skirt treatment effluent plume dynamics.

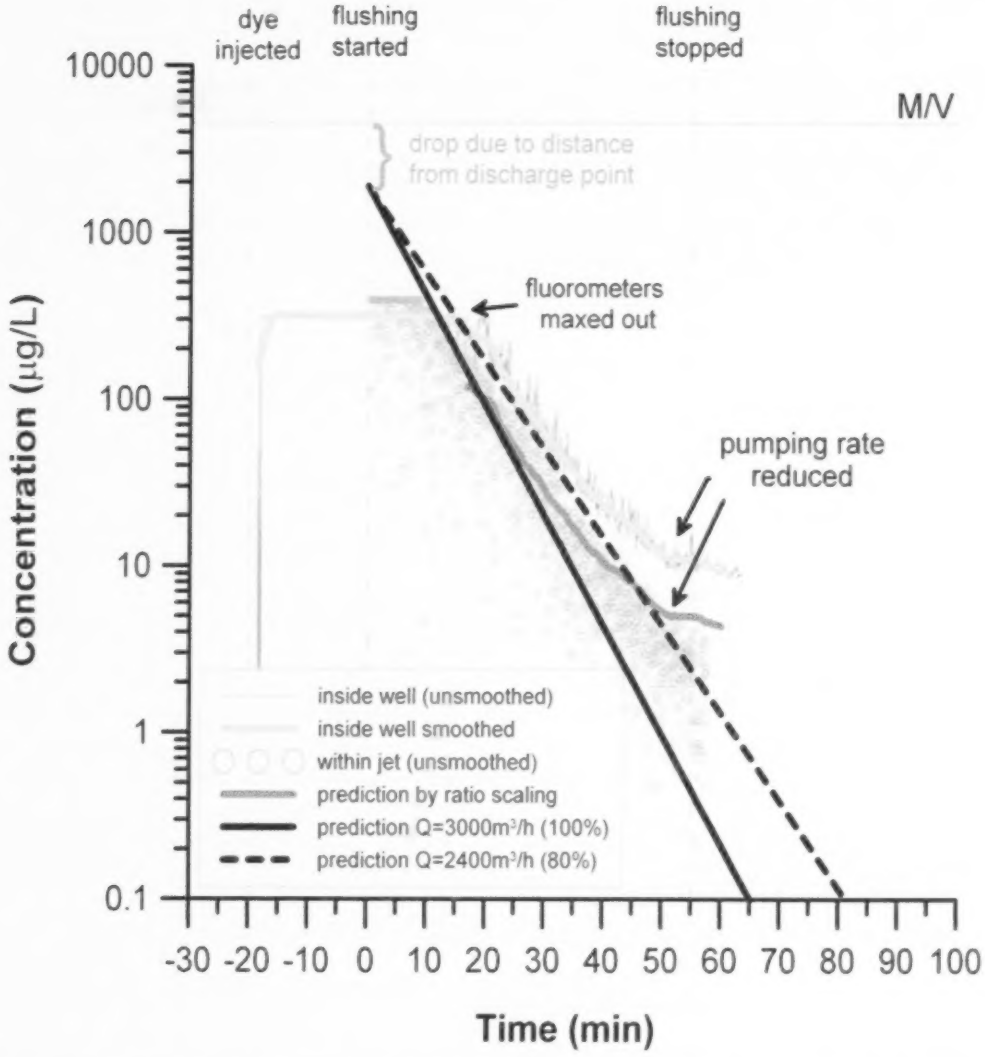

Figure 5: Time series of observed and predicted dye concentrations within the well and flushing discharge jet at a depth of 0.5 m and distance of 6 m from the discharge pipe. The straight line predictions (heavy solid and dashed black lines) are based on well flushing and jet flow dynamics. The heavy red line is a scaled version of the smoothed observations taken from within the well (green line, above). The blue line is the time series of observations taken from within the well. The open black circles are the unsmoothed dye concentration data. The M/V gridline is the calculated concentration of dye within the well prior to flushing.

Well-Boat Treatment: Dispersal Models

Although there is relatively good agreement between the Okubo model predictions and measured dye concentrations in the well-boat discharge plume, there is a tendency for the model predicted dye concentrations to exceed the observed concentrations at the lower end of the observed range. Since the dye concentration used in well-boat studies are at the lower end of the range of observed values, the Okubo model tends to overpredict dye concentration (Figure 6).

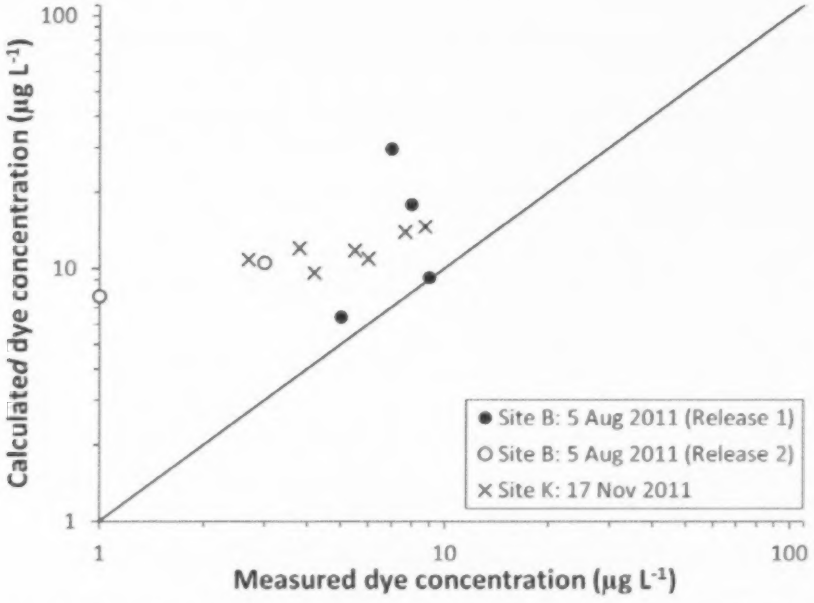

Figure 6: Comparison of measured and predicted dye concentrations in dye plumes resulting from well-boat treatments.

Well-boat Treatment: Factors Influencing Exposure

The factors influencing the concentration of pesticide released following well-boat bath treatments include the:

- volume of wells;
- angle of discharge (i.e., horizontal, vertical or at some other angle);
- diameter of the discharge pipe;
- depth or height of the discharge pipe below (or above) the sea surface;
- maximum rate of discharge flow (i.e., pumping capacity);
- mass of pesticide introduced into well (i.e., concentration);
- density of the discharge solution;
- velocity of discharge;
- direction of the discharge (i.e., into net-pens or away from net-pens);
- duration of the discharge;
- proximity of other net-pens and other farm infrastructure; and
- degree of bio-fouling on adjacent net-pens.

Environmental factors also influence the dilution of pesticide following well-boat bath treatments, such as:

- rates of ambient horizontal and vertical mixing in the receiving environment;
- proximity of vertical boundaries in relation to vertical stratification, the sea bottom and inter-tidal zones;
- proximity of horizontal boundaries such as the shoreline, bottom and pycnocline;
- weather, wind and waves; and
- water characteristics such as temperature, salinity, pH, dissolved oxygen content, suspended organic and sediment loads that may influence the chemical behaviour of the pesticide in the ambient water.

2) What are the known biological effects of Interox-Paramove® 50, Salmosan® 50WP, Excis®, and AlphaMax® on key non-target organisms?

Interox-Paramove® 50

Interox-Paramove® 50 (active ingredient: hydrogen peroxide) has a prescribed treatment concentration of between 1.2 x 10^6 µg/L to 1.8 x 10^6 µg/L (1800 mg/L) (as active ingredient) for 20–30 minutes depending on water temperatures.

Laboratory 1-hour Paramove® 50 LC_{50}s for adult American lobster (*Homarus americanus)* is >3.75 x 10^6 µg/L hydrogen peroxide (or >3750 mg/L), which is greater than the 1.2 x 10^6 µg/L - 1.8 x 10^6 µg/L hydrogen peroxide treatment concentration. The 1-hour LC_{50} for American lobster stage I is 1.637 x 10^6 µg/L hydrogen peroxide, for *Crangon* shrimp it is 3.182 x 10^6 µg/L hydrogen peroxide, *Mysid* shrimp it is 0.973 x 10^6 µg/L hydrogen peroxide (a 1.2-fold dilution), and for the copepods, *Acartia sp.,* it is 0.059 x 10^6 µg/L hydrogen peroxide, or a 20-fold dilution.

Morbidity was observed in non-parasitic copepods in the laboratory at concentrations as low as 0.012 x 10^6 µg/L hydrogen peroxide. The concentration at which 50% of the test organisms showed an effect, in this case a change in feeding, (EC_{50} (feeding)) was 0.0042 x 10^6 µg/L hydrogen peroxide (or 0.3% of treatment dose).

Salmosan® 50WP

Salmosan® 50WP (active ingredient: azamethiphos) is prescribed at 100–150 µg/L (as active ingredient) for 30–60 minutes (depending on temperature). Azamethiphos is a highly soluble chemical that is not predicted to bioaccumulate or persist.

Laboratory 1-hour Salmosan® 50WP LC_{50}s for American lobster adults is 24.8 µg/L azamethiphos, a four-fold dilution of the 100 µg/L azamethiphos treatment concentration. No 1-hour LC_{50} could be determined for American lobster stage I, *Crangon* or *Mysid* shrimp, as 50% mortality was not observed at the highest concentrations tested in the bioassay, 86.5, 85.5 and 85.5 µg/L azamethiphos, respectively.

In laboratory studies, adult female American lobsters were more susceptible to azamethiphos in the summer.

Bi-weekly exposures of pre-spawning females for up to six weeks at low concentrations (10 µg/L azamethiphos) for durations of 1-hour resulted in significant mortality (40%-100%) of animals exposed. Some survivors of exposures to 10 µg/L azamethiphos and other exposed to 5 µg/L azamethiphos, failed to spawn.

Multiple exposures (up to six exposures of 30 minute durations) to adult lobsters over three days with a maximum of 2 exposures per day at 0.1 µg/L or 1.0 µg/L azamethiphos resulted in no significant mortality and no observed effects on behaviour, molting or reproduction.

Repeated short-term exposures (15-120 minutes three times per day for three days) of adult lobsters to Salmosan® 50WP in concentrations below 25 µg/L azamethiphos resulted in up to 80% mortality and hyperactivity, disorientation, and paralysis in survivors. A No Observable Effects Concentration (NOEC), based on the results from the lethality studies, was estimated to be approximately 1 µg/L following 9 exposures of 2-hour duration over three days.

Chronic exposure to low concentrations of azamethiphos induces sublethal effects at the biochemical level in adult lobsters, which persist for at least 24 hours after cessation of exposure, thus increasing the risk of cumulative impacts when lobster are exposed to further chemical or non-chemical stress. Sublethal exposure markedly increases the risk of mortality of adult lobsters during simulated live transportation.

No effects on morbidity and mortality were observed in non-parasitic copepods exposed in the laboratory to concentrations as high as 500 µg/L azamethiphos.

No mortality was observed in other indigenous southwestern New Brunswick species, such as green crab, scallops, and soft-shelled clams in laboratory lethal toxicity tests at 100 µg/L azamethiphos.

Excis®

Excis® (active ingredient: cypermethrin) is a synthetic pyrethroid pesticide, that is not available in Canada for use on salmon farms. It is prescribed at 5 µg/L (as active ingredient) for 1-hour. As with other synthetic pyrethroids, cypermethrin has low water solubility, and can be expected to persist in the sediments where it might affect sediment-dwelling organisms or subsequently desorb and affect benthic invertebrates.

There is limited 1-hour exposure toxicity data available for Excis®. However, 24-hour Excis ® LC_{50}s for adult American lobster is 0.14 µg/L cypermethrin, a 35-fold dilution of the 5 µg/L cypermethrin treatment concentration, while the 12-hour LC_{50} for American lobster stage II is reported as being as low as 0.058 µg/L cypermethrin (an 86-fold dilution; from Pahl and Opitz, 1999). The 1-hour Excis® LC_{50} for *Mysid* shrimp is >0.142 µg/L cypermethrin (<35-fold dilution).

Repeated short-term exposures (15 - 120 minutes three times per day for three days) of adult American lobsters to up to 1.8 µg/L cypermethrin resulted in up to 80% mortality and disorientation and paralysis in survivors. The NOEC, based on lethality, was estimated to be 0.025 µg/L (as cypermethrin) based on 9 exposures of 60 minutes duration over three days.

No mortality was observed in non-parasitic copepods exposed in the laboratory to 5 µg/L cypermethrin, but morbidity was observed at 5 µg/L and 0.5 µg/L cypermethrin. The EC_{50} (feeding) was estimated to be 0.297 µg/L cypermethrin.

AlphaMax®

AlphaMax® (active ingredient: deltamethrin) is prescribed at a treatment concentration of 2 µg/L (2000 ng/L) (as active ingredient) for 30 minutes. Deltamethrin is a synthetic pyrethroid with low water solubility, and can be anticipated to persist in the sediments where it may affect sediment-dwelling organisms or may subsequently desorb and affect benthic invertebrates.

Laboratory 1-hour AlphaMax® LC_{50}s for American lobster adults is 0.0188 µg/L (18.8 ng/L) deltamethrin, a 110-fold dilution of the 2 µg/L deltamethrin treatment concentration. The 1-hour LC_{50} for American lobster stage I is 0.0034 µg/L deltamethrin (590-fold dilution), *Crangon* shrimp is 0.142 µg/L deltamethrin (14-fold dilution), and *Mysid* shrimp is 0.0139 µg/L deltamethrin (140-fold dilution). The LC_{50} for the west coast amphipod, *Eohaustorius estuarius*, is 0.0131 µg/L deltamethrin (a 150-fold dilution).

Multiple exposures (up to six exposures for 30 minute durations) to adult American lobsters over three days, with a maximum of 2 exposures per day, at 0.002 µg/L or 0.020 µg/L deltamethrin resulted in mortality from 0-5% at 0.002 µg/L deltamethrin, and from 0-10% mortality at the 0.020 µg/L deltamethrin, but mortality was not related to number of exposures. No effects on molting or reproduction were observed in surviving adult lobsters.

No mortality was observed in non-parasitic copepods exposed, in the laboratory, to 2 µg/L of deltamethrin, but morbidity was observed at 2 µg/L and 0.2 µg/L deltamethrin (treatment concentration and 10% of treatment concentration). EC_{50} (feeding) was estimated at 0.040 µg/L deltamethrin.

Table 1 summarizes the lowest concentration of active ingredient for each of the four anti-sea lice pesticides formulations that resulted in at least one death or noted behavioural response during the 1-hour lethality studies.

Table 1. Lowest concentration (µg/L) of active ingredient for four anti-sea lice pesticide formulations (active ingredient, treatment concentration) for 1 hour exposures that resulted in at least one death or noted behavioural responses during lethality studies.

Species/Life Stage	Salmosan® (azamethiphos 100 µg L^{-1})	AlphaMax® (deltamethrin 2 µg L^{-1})	Excis® (cypermethrin 5 µg L^{-1})	Paramove 50® (hydrogen peroxide 1.2 $x10^6$ – 1.8 $x10^6$ µg L^{-1})
American Lobster Stage I	11.1	0.62	NA	1.875×10^5
American Lobster Adult	3.7	0.017	NA	7.50×10^5
Crangon septemspinosa	1.2*	NA	NA	3.75×10^5
Mysis sp.	NA	0.002	0.18	NA

** lowest exposure concentration in bioassay.*

3) What is the potential for non-target organisms to be exposed to anti-sea lice pesticides, post-treatment, at biologically relevant concentrations?

In order to give an indication of the combination of pesticide concentration, *in situ* exposure duration and the predicted associated biological effects at this exposure, the aaverage treatment and effluent concentrations were divided by the level of effect (LC_{50}) concentration, resulting in a risk quotient. Using this approach, an indication of potential risks associated with exposure and biological effects for the four pesticides, different applications and the selected indigenous non-target species and life-stages can be estimated. In standard risk procedures, risk quotients greater than one (RQ>1) indicate that there is potential for adverse effects.

For Paramove® 50 at treatment concentration, the risk quotient ranges from <0.3 – 1.2 for American lobster larvae stage I, adult American lobsters, *Crangon*, and *Mysid* shrimp, based on 1-hour LC_{50} data. For Salmosan® 50WP at treatment concentration, the risk quotient ranges from <1 – 3.1 for American lobster larvae stage I, adult American lobsters, *Crangon*, and *Mysid* shrimp, based on 1-hour LC_{50} data. For Excis® at treatment concentration, the risk quotient for copepods is 35 based on 1-hour LC_{50}. For AlphaMax® at treatment concentration, the risk quotient ranges from 14-588 for lobster larvae stage I and III, adult lobsters, *Crangon*, and *Mysid* shrimp, based on 1-hour LC_{50} data.

Release Following Net-Pen Treatment

Building on the transport and dilution analysis based on the modified Okubo model, once a pesticide is released from net-pens following tarp application, it is estimated to be diluted, on average, by a factor of 10 times in 30 min, 100 times in 1 hour, and 1000 times in 3 hours. For Salmosan® 50WP, application of the model suggests that dilution from treatment concentration to a risk quotient of 1 (equal to 1-hour LC_{50}) takes about 10 minutes and the plume area is estimated to be approximately 1100 m^2. The pesticide that has shown the highest toxicity, AlphaMax®, is estimated to take up to 3 hours to dilute to a 1-hour LC_{50}, for American lobster larvae stage I, which is the most sensitive species and life stage tested for this product. At this point, the size of the pesticide plume is estimated to be approximately 50,000 m^2 and to have traveled several kilometres away from the treatment location.

Operationally, multiple net-pens within a farm may be treated per day, but the number of treatments will be limited by label conditions for the pesticide and operational and environmental constraints. In order to estimate what the effect of multiple treatments may be on non-target organisms, two different scenarios were examined: (1) multiple net-pens treated in essentially the same area, resulting in an overlap in the pesticide effluent plume and (2) multiple net-pens treated but without the effluent plumes overlapping, resulting in a larger area being affected.

Modeling approaches using an implementation of FVCOM can be used to estimate the total potential area that may be impacted by multiple treatments. Alternatively, an estimated upper limit of the potential area exposed by multiple treatments may be obtained by assuming a circle of radius equal to the predicted transport distance, using the modified Okubo model. These circles can be centred on farm sites and plotted on a map to provide an estimate of the horizontal spatial domains that are of potential risk to exposure by a particular pesticide. The

transport distances, based on the risk quotients and the approximate length scales calculated using the modified Okubo model are 100 m – 500 m for Salmosan® 50WP, 500 m – 1000 m for Excis® and 1000 m – 5000 m for AlphaMax®.

Based on the maximum depth of vertical mixing observed during dye/treatment applications (<25 m), farms sited in areas of average low water depth less than or equal to 20 m are more likely to experience benthic exposure to pesticides following treatments. The theoretical models for predicted vertical mixing is less certain than for horizontal mixing; more work will be required to refine information on vertical mixing.

Release Following Well-boat Treatment

Once treatments are completed within well-boats, the treatment water is flushed out and fresh seawater is simultaneously pumped into the treatment well. The concentration of pesticide within the discharged water will vary with the pumping rates used. Operationally, there is an effort to achieve maximum pumping rates. The well-boats currently operating in southwestern New Brunswick aim to flush at approximately 2400 m^3/h. For Paramove® 50, this results in a risk quotient ranging from 0.12 to 0.46, for American lobster stage I, adult American lobster, *Crangon*, and *Mysid* shrimp. For Salmosan® 50WP the risk quotient ranges from 0.38 to 1.16, for American lobster stage I, adult American lobster, *Crangon*, and *Mysid* shrimp. For AlphaMax® the risk quotient ranges from 5.3 to 221 for American lobster stage I, adult American lobster, *Crangon*, and *Mysid* shrimp, based on 1-hour LC_{50}s.

Once the effluent is pumped out of the well-boat, dilution occurs from entrainment of water into the effluent, such that at 10 m from the discharge point, the concentration will be reduced to 25% of the initial discharge concentration, and by 100 m there will be a $1/40^{th}$ dilution in the concentration of each pesticide, and therefore a decrease in the calculated risk quotients. For example, for American lobster larvae stage I, based on 1-hour LC_{50} the risk quotient for Alphamax® at discharge concentration is 221, by 10 m it is reduced to 55, and by 100 m it is reduced to 6. This reduction is dependent on the diameter of the discharge pipe and therefore may change with different well-boat configurations.

Potential Exposure and Effects related to Life-cycle of Non-Target Organisms

Sea lice treatment may take place in southwest New Brunswick from April through to the end of December, depending on sea lice pressures and water temperatures. Potential exposure to pesticides that may be used to treat sea lice will also depend on whether the non-target organisms are present at the time of treatment and in the area of treatment. While this analysis did not consider population dynamics of non-target organisms, the selected non-target organisms considered in the toxicity studies are known to be present in the Bay of Fundy, as per Table 2. The migratory patterns and seasonality of location of non-target organisms need to be considered as part of the overall risk of exposure to pesticides, and incorporated into integrated pest management approaches.

Table 2. Location and seasonal distribution of invertebrate species native to southwest New Brunswick that have been tested for their sensitivity to anti-sea lice pesticides. The bulk of sea lice treatments take place from April to December, depending on water temperature.

	Larval lobsters Stages I-III	Juvenile lobsters Stage IV +	Adult lobsters	Mysids	Crangon
Position in water column	Pelagic	Subtidal, benthic	Subtidal, benthic, epi-benthic	Intertidal, subtidal, pelagic or epi-benthic, depending on species, habitat, and time of year	Intertidal, subtidal, epi-benthic
Presence in Bay of Fundy	June - Sept	Year-round	Mobile, but present year round	Seasonal	Year-round

4) Can the dispersion models and toxicity studies be applied to other salmon farming areas in Canada and other non-target species?

The methods that are available for the application of anti-sea lice pesticides are the same in all salmon farming areas of Canada.

Generally, the methods and principles developed for studying pesticide discharges and their transport and dispersal in the marine environment in southwest New Brunswick can be applied in other salmon farming regions in Canada. In particular the Okubo model, with modifications, can be used to predict the area that may potentially be exposed. To apply this modified model, specific inputs are required: the mass of product added and the mixing depth in the receiving environment must be specified. This model does not predict the velocity (speed and direction) of the discharge, nor the distance and direction away from the release site. It does allow estimation of dilution as a function of time post-release.

More sophisticated transport and dispersal models (e.g., FVCOM) require more inputs (winds, tides, bathymetry, freshwater inputs, etc.) and localized calibration. As these inputs will vary with local and regional differences in conditions, a model solution calculated for a particular time and place cannot be applied elsewhere, and each location will require data for validation. Ultimately, FVCOM will give a four-dimensional (X,Y,Z, and t) estimate of the receiving water currents which can be input into a particle tracking model which then predicts the movement and dilution of the discharge.

Regardless of the model used, the bathymetry of the location is necessary to predict whether discharge will reach the bottom.

By integrating the ranges in plume area at the end of the dilution time to reach 1-hour LC_{50}, the bathymetry, the depth of vertical mixing observed (<25 m) and site locations, an assessment of the potential for benthic interaction with the released pesticide and the potential for overlaps in discharge plumes between farms can be made (Figure 7).

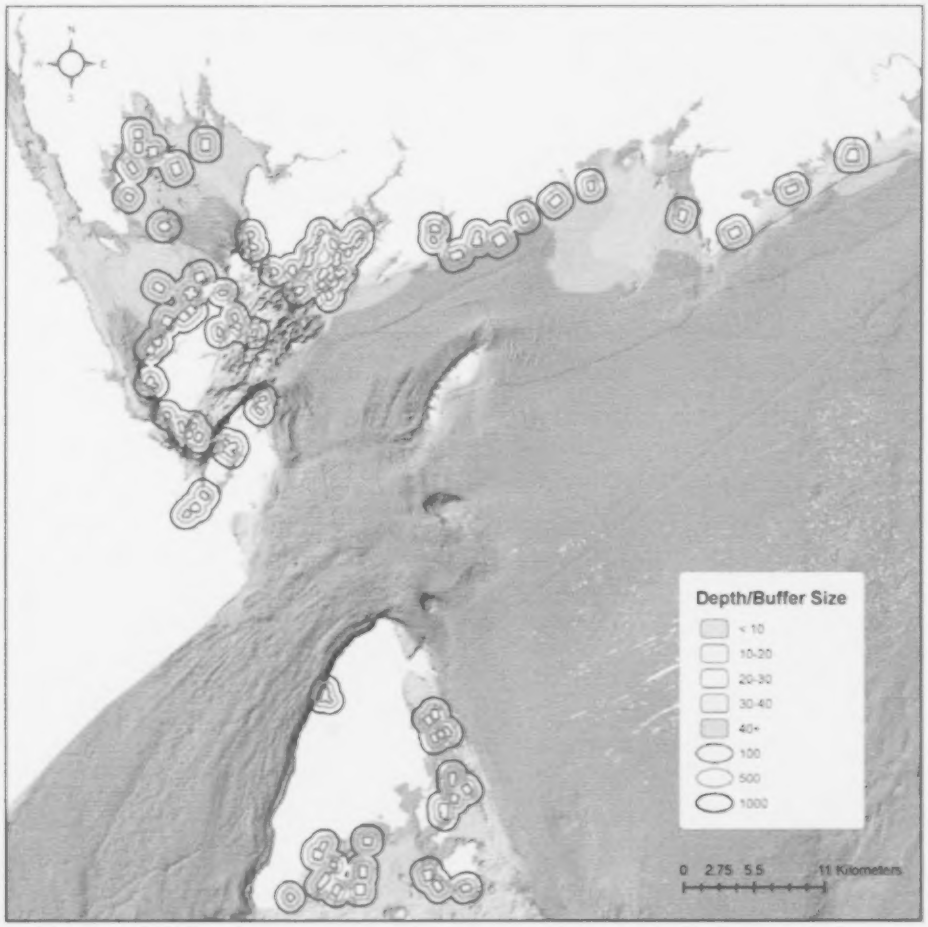

Figure 7: Map showing the location of fish farm sites in relation to the bathymetry of the southwest New Brunswick coastal area, and the 100, 500 and 1000 m zones of influence.

Differences in environmental conditions over time and between sites/regions will influence the fate and toxicity of pesticides in the marine environment. For example, temperature plays a role in the toxicity of the pesticide, the physiology of organisms, and the rate at which pesticides breakdown. The amount of organic matter in the water column can also affect the toxicity of pesticides and how they are distributed in aquatic environments.

The methods used to study the toxicity of pesticides to non-target species can be applied to species found in other regions of Canada. A Species Sensitivity Distribution analysis was not constructed as part of this analysis, although such a distribution has value as a predictive tool. For a specific species of interest, toxicological studies, where they do not already exist, might have to be carried out.

The method developed to estimate risk using predicted exposure and toxicity information can be applied across all regions. However, risk characterization will require local knowledge of the non-target species (e.g., presence/abundance and population structure).

During this process, it was recognized that the approach developed predicts risk to individuals but does not address population scale risks. In addition, risk characterization should also consider risks from other activities. Although it is difficult to apportion risk associated with pesticide exposure from aquaculture activities relative to other sources of harm in the environment, the models developed could be applied to other activities with similar point source outputs.

Sources of Uncertainty

Uncertainties related to the factors that influence exposure

- The rate at which the pesticide is dispersed from the cage site after bath treatments, regardless of application, depends on the environmental, husbandry, biophysical and operational factors and is therefore site specific.
- The effect of the pumping rate from the well-boats on dilution of pesticide is a sensitive variable, and currently not well controlled. This variability may have impact on subsequent calculations or models that incorporate this information.
- More work will be required to refine information on vertical mixing and potential exposure of benthic environments.
- Validation of the modified Okubo model with different farm infrastructure configurations is required.
- Models are representations of reality using approximations and simplifications and as such contain uncertainties and are not an exact recreation of reality.
- The FVCOM hydrodynamic model for southwest New Brunswick does not accurately predict the size of plume post-release (tarp or well-boat).
- A relationship between the fluorescent dye used in the field studies and Interox-Paramove® 50 (hydrogen peroxide) was not established due to interactions between the product formulation that interfered with the ability to measure hydrogen peroxide concentration in the presence of the dye. Therefore, the nature of the relationship between the fluorescent dye and Interox-Paramove® 50 is unknown.

Uncertainties related to the biological effects on key non-target organisms

- Pesticide toxicity was based on laboratory studies with filtered sea water. The toxicity of aquaculture pesticides when raw seawater and sediment is present in the bioassay tests needs further study.
- There is uncertainty regarding the applicability of laboratory studies to what may occur in the field (e.g., exposure time, routes of exposure, and water quality).
- Short term acute toxicity tests do not account for any delayed effects on normal development (e.g., delayed lethality, reproductive impairment, etc.).

- Further studies are needed on the interactions between aquaculture pesticides and other stressors, such as hypoxia, emersion, temperature change and handling, which could lead to lethal impacts on crustaceans exposed to sublethal concentrations of pesticides.

- The effects of water temperature and physiological state of non-target organisms may influence sensitivity to pesticides.

- Limited laboratory toxicity data are available for non-target species indigenous to other salmon farming regions of Canada. While a Species Sensitivity Distribution analysis can provide some predictions on relative toxicity of a pesticide to other species, the specific toxicity effects of the four pesticides examined on other non-target species indigenous to other salmon farming regions in Canada that have not been tested is unknown.

- It is uncertain whether there will be a change in the toxicity to non-target organisms should there be a change in formulation of the pesticide.

- Limited analysis of the impacts of sequential exposure to different pesticides has been undertaken, and the potential for synergistic impacts to non-target organisms will require further study.

Uncertainties related to the concentration and duration, dispersion and biological effects

- The exposure to the plume of planktonic larval stages may not be accurately reflected by 1-hour exposure estimates because these stages may travel with the plume, resulting in a much longer exposure. Therefore, the 1-h LC_{50}-based risk quotient estimates may underestimate the effects, and longer duration LC_{50} data may be more appropriate, or a more conservative estimate could be derived from the use of NOEC data or sublethal data, particularly for pelagic organisms.

- The potential interactions between the discharged pesticides and the benthic environment is not well characterized, although an estimate based on the maximum exposure depth detected during field studies and the bathymetric and geographical information of present aquaculture sites in southwest New Brunswick provides an indication of potential interactions. Further information and assessment of potential interactions with the benthic environment, including fate of the pesticides, will be required to refine this estimate.

- The appropriate exposure duration for lethal toxicity testing (LC_{50}) of benthic organisms that will be representative of potential exposure in the field will depend, in part, on the pesticide in question and whether it binds to sediments. Water-borne exposure times for benthic organisms, such as adult lobsters, may be shorter or longer than the 1-hour exposure used in the LC_{50} testing, and will likely depend on local factors (i.e., bathymetry, currents, tides, etc.).

- Although the potential for exposure of selected non-target organisms has been identified based on a general knowledge of their presence in the area, further refinement in order to assess both the likelihood and extent of interactions will be required. A more comprehensive assessment of the movement, migration, and relative abundance of the

population and life stages will facilitate an evaluation of the likelihood of interaction with respect to the non-target species population in its entirety and, as required, as a fishery.

- Field validation of the effects of pesticide exposure post-treatment, through the use of field sentinel species studies, requires validation of exposure to the pesticide. This can be achieved through the use of dye mixed with the pesticide treatment, and attaching fluorometers to the location of sentinel species placed in the field.

Uncertainties related to the application of results to other salmon producing areas in Canada

- Toxicological data was only presented for one sensitive aquatic species native to British Columbia.

- Comparison of model outputs from the Okubo model (modified and unmodified) and the implementation of the FVCOM model have only been undertaken for southwest New Brunswick. Therefore, while the modified Okubo model should be applicable to other salmon producing areas in Canada, the relative accuracy of this and other models under these different local conditions, and in comparison to other internationally-implemented models of transport and dispersion has not been assessed.

CONCLUSIONS

Factors that Influence Exposure

There is generally a good relationship between dye concentration and pesticide concentration, therefore the dye concentration is a good surrogate for field pesticide concentrations, over time periods of a few hours.

Dye/pesticides were rarely well mixed during tarped net-pen applications in southwest New Brunswick, whereas they were usually well mixed in well-boats.

Dye/pesticide flushing time following release from tarped net-pens treatment ranged from minutes to hours and is influenced by currents, netting, bio-fouling, and other factors, which can change from site to site. Dye/pesticide flushing from well-boats is mechanically controlled and therefore occurs over a shorter period of time, and is associated with simultaneous flushing and dilution.

For tarp applications, the unmodified Okubo model based-approach gives an underestimate of pesticide dilution from net-pens over the first few hours after release. However, the modified Okubo model based-approach takes into consideration the initial enhanced horizontal spread (assumed to be due to cage infrastructure) and gives a more accurate estimation of the concentration and size of the effluent plume.

The characteristics of the immediate discharge from well-boats seem to be reasonably well represented by jet dynamic steady state theory. Entrainment of seawater into the effluent results in additional dilution of the pesticide up to a factor of 40 at a distance of 100 m from the discharge point based on the diameter of the discharge pipe.

Although the work was conducted in southwest New Brunswick, the general principles and orders of magnitude dilution are expected to apply elsewhere. The unmodified Okubo relationship is based on data collected from many places around the world and it should give a conservative estimate of concentrations in other areas of Canada as well. The modified version should be validated over a broader range of locations. Differences from location to location are the local hydrography, bathymetry and perhaps treatment procedures. The local stratification and current regime will dictate the depth to which pesticides mix and the direction and magnitude of the pesticide transport. The bathymetry within the zones of influence will dictate whether the benthic habitat will be exposed.

The dilution of pesticide, post-treatment, to 1-hour LC_{50} concentrations occurs more quickly and over a smaller area following well-boat application compared to tarp application. This is due to the small volume of water in the treatment wells, resulting in smaller quantities of pesticides used per treatment and mechanical dilution through flushing and entrainment of water into the pesticide effluent.

The primary factors that influence the transport and dispersal of the pesticide, post-treatment are:

- Operational or husbandry factors such as the:
    - quantity and degradation properties of pesticide used;
    - influence of net-pen and farm infrastructure on initial dispersal;
    - net mesh size and bio-fouling (i.e., porosity); and
    - proximity of other net-pens and other farm infrastructure.
- Environmental factors, including:
    - rates of ambient horizontal (x,y) vertical mixing in the receiving environment;
    - proximity of vertical boundaries in relation to vertical stratification, the sea bottom and inter-tidal zones;
    - proximity of horizontal boundaries such as the shoreline, bottom and pycnocline;
    - weather, wind and waves; and
    - chemical behaviour of the pesticide in the ambient water (density, decay, absorption, etc.).
- Location:
    - location-specific flows and mixing;
    - proximity of bottom and shoreline, and
    - the distribution of sensitive non-target organisms.

Biological Effects

The treatment concentration for the four pesticides differs over orders of magnitudes, which reflects the relative toxicity of each pesticide. Some pesticides require a greater dilution in order to reach a no effects level.

Laboratory studies of the four pesticides using 1-hour LC_{50} for lobsters and other indigenous invertebrates, confirm that the relative toxicity and associated potential magnitude of effects increases from Interox-Paramove® 50 (active ingredient: hydrogen peroxide) to Salmosan® 50WP (active ingredient: azamethiphos) to Excis® (active ingredient: cypermethrin) to

AlphaMax® (active ingredient: deltamethrin). The sensitivity of non-target species to each of the four pesticides is species and life stage dependent.

Of the four pesticides studied, there is considerably more toxicology data available for Salmosan® 50WP.

Effects (lethal and sub-lethal) have been observed for all pesticides tested on select non-target species in the lab at concentrations below the prescribed treatment concentration.

Chronic exposure to low concentrations of azamethiphos induces sublethal effects at the biochemical level in adult lobsters, which persist for at least 24 hours after cessation of exposure, thus increasing the risk of cumulative impacts when lobster are exposed to further chemical or non-chemical stress. Sublethal exposure markedly increases the risk of mortality of adult lobsters during simulated live transportation.

In multiple exposure studies, when adult lobsters were exposed up to 9 times (15 - 120 minutes, three times per day for three days) to a range of azamethiphos concentrations (0.5 - 25% of recommended treatment concentration) up to 80% died and hyperactivity, disorientation, and paralysis were observed in some survivors. A NOEC (lethality) level of 1.03 µg/L (as azamethiphos) when exposed repeatedly for 120 minute was estimated from these data. When adult female lobsters were exposed to 10 µg/L for 1 hour biweekly (n = 4 exposures) a significant number of lobsters died and spawning was affected in some survivors. However, when adults were exposed (up to six exposures for 30 minute durations) over three days with a maximum of 2 exposures per day at 0.1 µg/L or 1.0 µg/L no significant mortality and no effects on behaviour, molting, and reproduction was observed.

AlphaMax® is the most lethal pesticide tested, and requires the highest dilution to reach non-lethal concentrations.

Exposure and Effects

Following the application of anti-sea lice pesticides, the resulting effluent will become diluted and distributed away from the discharge site. Models to predict the extent of dilution and location of the resulting effluent can provide some indication of potential exposure to a given aquaculture pesticide. When combined with toxicity data and life-history information for sensitive non-target species, a risk quotient can be calculated. Validation of modeled exposure estimates requires measurement of environmental concentrations and durations.

Based on the estimated lethal concentration risk quotient for the most sensitive non-target species tested, the pesticide that has the lowest risk quotient at treatment concentration is Interox-Paramove® 50 (RQ=1.2), followed by Salmosan® 50WP (3.1), Excis® (151) and AlphaMax® (588).

The predicted area of influence has also been calculated for each pesticide once the risk quotient equals the LC_{50} for the specific non-target organism. This area, for the most sensitive non-target species tested, following tarp treatment, ranges from approximately 0 m^2 for Interox-Paramove® 50, 1100 m^2 for Salmosan® 50WP, and 50,000 m^2 for Alphamax®. 1-hour LC_{50} data was not available for the range of non-target organisms for Excis®.

Because of the jet plume steady state dynamics and subsequent entrainment of the discharge plume following pesticide treatment in a well-boat, calculating the area of influence is more complicated. However, the amount of pesticide required per well-boat treatment is less than per tarp treatment, but multiple well-boat treatments may be required to treat the total biomass of fish within a net-pen.

Risk quotients were calculated for the well-boat discharge concentration and following dilution due to entrainment of ambient seawater at 10 and 100 m. The resulting estimated risk quotients for the well-boat discharge concentration, for the most sensitive species tested, based on 1-hour LC_{50}s, ranged from 0.46 for Interox-Paramove® 50, to 1.16 for Salmosan® 50WP, and 221 for AlphaMax®. At a distance of 10 m from the discharge pipe, the risk quotients are calculated to be 0.1 for Paramove® 50, 0.3 for Salmosan® 50WP, and 55 for AlphaMax®. At a distance of 100 m from the discharge pipe, the risk quotients ranged from 0.01 for Interox-Paramove® 50, 0.03 for Salmosan® 50WP and 6 for AlphaMax®.

The predicted area of influence following well-boat treatment will be less than the area of influence following tarp treatment. This is concluded, in part, because the calculated risk quotient values for the well-boat discharge concentration are lower than the estimated ratios for tarp treatment concentration, which is the equivalent to discharge concentration for tarp treatments, and due to the anticipated dilution associated with entrainment of seawater associated with the well-boat discharge.

This analysis only applies to those animals that exist in the water column and therefore does not include consideration of benthic exposure.

Application to Other Salmon-Producing Areas in Canada

Generally, the methods and principles developed for studying pesticide discharges and their fate in the marine environment in southwest New Brunswick can be applied in other salmon farming regions in Canada. However, to apply these models and to improve their predictability local oceanographic and environmental data is required for model parameterization.

The method developed to estimate risk of pesticide exposure to non-target organisms can be applied across all salmon farming regions in Canada. However, risk characterization will require local knowledge of the biology, ecology and population dynamics of non-target species

OTHER CONSIDERATIONS

The question of target dose was raised as a factor that could influence the exposure analysis. Analytical laboratory results from field water samples following Salmosan® or AlphaMax® treatment report concentrations well below target. This is considered to be a factor that requires further study, including laboratory QA/QC confirmation of analytical results.

If corroborated, lower treatment concentrations will influence both exposure concentration and predictions for the non-target organisms in this study, as well as sea-lice treatment efficacy, and the potential for the development of resistance by sea lice to the pesticides in question. Thus, label concentrations have to be taken into account to ensure exposure model accuracy for approved pesticides.

The oceanographic models presented, particularly those based on the FVCOM model are resource intensive for data collection, quality control, entry, model optimization and execution.

There are other anti-sea lice pesticides under development or used outside Canada, that likely lack Canadian-specific toxicological and fate data in the marine environment which may be required to support environmental risk assessments under the Pest Control and Products Act. Pro-active research by industry seeking additional treatment options as part of an integrated pest management plan was raised as being potentially beneficial.

Since it is resource-impossible to develop site-specific models for all salmon farming sites in Canada, it is important to consider extrapolation to "like-sites". This will necessitate delineation of the key environmental (physical, chemical and biological) factors, as well as non-target species subject to the question of risk; which make them "comparable". As above, this will help streamline resources applied to exposure-toxicology risk assessments – i.e., triage of sites that are distinct enough to warrant specific attention.

Monitoring, as a key component of pesticide treatment protocols is necessary, not only to address risk-assessment uncertainties (e.g., cumulative effects), but also to address uncertainties related to changes in the spatial and biological treatment 'footprint' environment (inter alia species expansion, changes in distribution, spawning times/locations, temperature, salinity and pH) that may impact pesticide chemical dynamics or species at risk of exposure.

SOURCES OF INFORMATION

This Science Advisory Report is from the March 13-15, 2013 assessment of Guidelines on Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments: Anti-Sea Lice Bath Treatments (Part II). Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

Okubo, A. 1971. Oceanic diffusion diagrams. Deep-Sea Research 18: 789-802.

Okubo, A. 1974. Some speculations on oceanic diffusion diagrams. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 167: 77-85.

Pahl, B.C., and Opitz, H.M. 1999. The effects of cypermethrin (Excis) and azamethiphos (Salmosan) on lobster *Homarus americanus* H. Milne Edwards larvae in a laboratory study. Aquaculture Res. 30: 655-665.

THIS REPORT IS AVAILABLE FROM THE:

Canadian Science Advisory Secretariat
National Capital Region
Fisheries and Oceans Canada
200 Kent Street
Ottawa, Ontario K1A 0E6

Telephone: (613) 990-0293
E-Mail: csas-sccs@dfo-mpo.gc.ca
Internet address: www.dfo-mpo.gc.ca/csas-sccs/

ISSN 1919-5087
©Her Majesty the Queen in Right of Canada, 2013

Correct Citation for this Publication:

DFO. 2013. Potential exposure and associated biological effects from aquaculture pest and pathogen treatments: anti-sea lice pesticides (part II). DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2013/049.

Aussi disponible en français:

MPO. 2013. Exposition potentielle et effets biologiques connexes issus des traitements des parasites et des agents pathogènes en aquaculture : pesticides contre le pou du poisson (partie II). Secr. can. de consult. sci. du MPO, Avis sci. 2013/049.